



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



ACE2, B⁰AT1, and SARS-CoV-2 spike protein: Structural and functional implications

Yuanyuan Zhang^{1,2}, Renhong Yan³ and Qiang Zhou^{1,2}

Abstract

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has emerged as a public health crisis and led to tremendous economic devastation. The spike protein (S) of SARS-CoV-2 hijacks the angiotensin converting enzyme 2 (ACE2) as a receptor for virus entry, representing the initial step of viral infection. S is one of the major targets for development of the antiviral drugs, antibodies, and vaccines. ACE2 is a peptidase that plays a physiologically important role in the renin–angiotensin system. Concurrently, it also forms dimer of heterodimer with the neutral amino acid transporter B⁰AT1 to regulate intestinal amino acid metabolism. The symptoms of COVID-19 are closely correlated with the physiological functions of ACE2. In this review, we summarize the functional and structural studies on ACE2, B⁰AT1, and their complex with S of SARS-CoV-2, providing insights into the various symptoms caused by viral infection and the development of therapeutic strategies.

Addresses

¹ Center for Infectious Disease Research, Westlake Laboratory of Life Sciences and Biomedicine, Key Laboratory of Structural Biology of Zhejiang Province, School of Life Sciences, Westlake University, 18 Shilongshan Road, Hangzhou 310024, Zhejiang Province, China

² Institute of Biology, Westlake Institute for Advanced Study, 18 Shilongshan Road, Hangzhou 310024, Zhejiang Province, China

³ Department of Biochemistry, School of Medicine, Southern University of Science and Technology, Shenzhen 518055, China

Corresponding authors: Zhou, Qiang (zhouqiang@westlake.edu.cn); Yan, Renhong (yanrh@sustech.edu.cn)

Current Opinion in Structural Biology 2022, 74:102388

This review comes from a themed issue on **Membranes**

Edited by **Anirban Banerjee** and **Manuel Palacín**

For complete overview of the section, please refer the article collection - [Membranes](#)

Available online 20 April 2022

<https://doi.org/10.1016/j.sbi.2022.102388>

0959-440X/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

Introduction

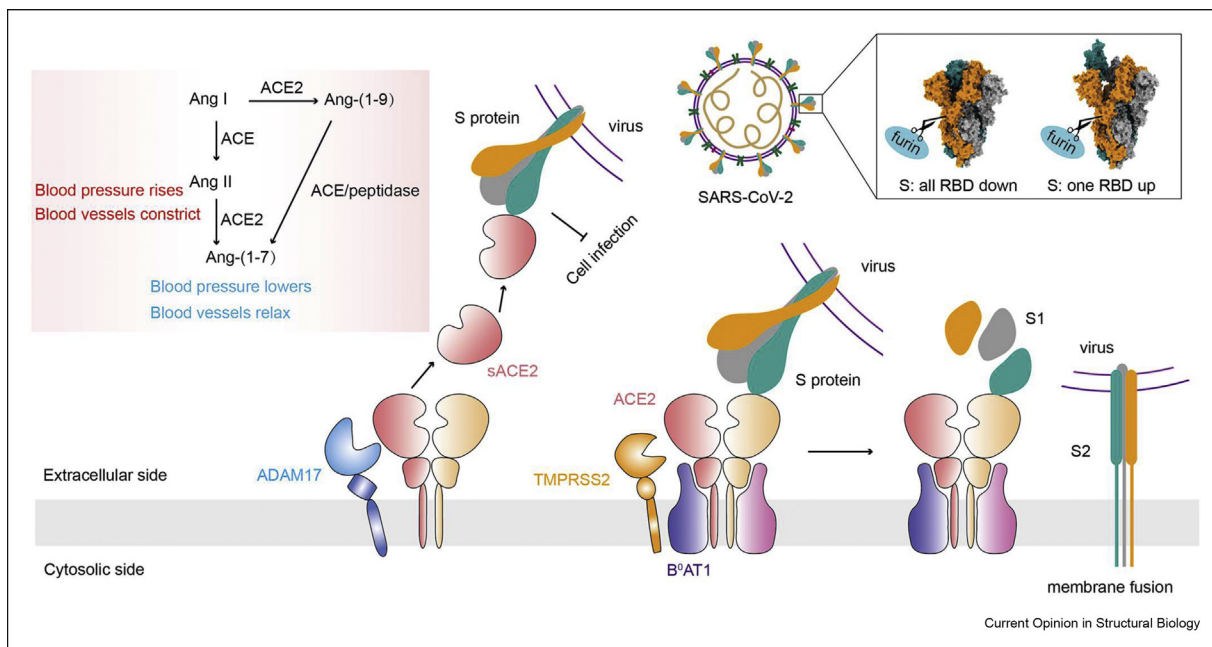
The emergence and continued presence of SARS-CoV-2 variants highlight the need to develop effective

interventions against COVID-19 pandemic. S of SARS-CoV-2 [1,2] is responsible for receptor recognition and membrane fusion, similar to that of SARS-CoV-1, which has caused the severe acute respiratory syndrome pandemic in 2002–2003 [3]. During assembly of the virus, S is cleaved into the S1 and S2 subunits by furin or furin-like proprotein convertase [4]. The S1 subunit binds to the viral entry receptor ACE2 through the receptor binding domain (RBD) [5] (Figure 1). Then, S undergoes second protease processing to release the membrane fusion peptide by transmembrane protease serine 2 (TMPRSS2) [6,7] or *cathepsin L* [8] on S2' cleavage site to mediate the fusion of the virus and host cell membrane [9]. The interaction between S and ACE2 is the important step of viral infection, therefore making it the critical target for developing the small molecule drugs, neutralizing antibodies and vaccines [10,11].

The lung damage followed by pulmonary fibrosis and chronic impairment of lung function is one typical symptom for SARS-CoV-2 infection [12]. In addition to its role as the receptor for SARS-CoV-2 invasion, ACE2 is a peptidase belonging to the renin–angiotensin system that controls vasoconstriction and blood pressure in human [13]. The *ace2* gene knockout mice showed that the downregulation of ACE2 significantly increases angiotensin II (Ang II) levels in the lungs and plasma, causing acute lung failure [14]. In addition, Ang II levels in the lung tissues of the mice were significantly increased after treatment with acid and SARS-CoV-1 Spike-Fc (a fusion protein of SARS-CoV-1 S with the Fc portion of human IgG1), and lung damage induced by this treatment could be attenuated by blocking Ang II receptor type 1 (AT1R) using its inhibitor [15]. These results collectively suggest that SARS-CoV-2 infection is closely correlated with the primary physiological function of ACE2. Besides, ACE2 forms heterodimers with the intestinal transporter B⁰AT1 to mediate the uptake of neutral amino acids, which provides an important insight into enterocyte infection with SARS-CoV-2 [16,17].

In the past few years, many studies have reported on SARS-CoV-2 and its receptor, ACE2. In this review, we will focus on the physiological functions and structural information of ACE2, B⁰AT1 and their complex with S of SARS-CoV-2, which can help us understand the

Figure 1



Schematic diagram of multi-functional ACE2. ACE2 plays a major role in the renin–angiotensin system by cleaving Ang II to Ang-(1–7) or Ang I to Ang-(1–9) (pink inset), which can decrease blood pressure and relax blood vessels. ACE2 is also a chaperone for the neutral amino acid transporter B^oAT1 in small intestine. Besides, ACE2 acts as a receptor for multiple viruses, such as SARS-CoV-2 or SARS-CoV-1. Dozens of S are localized on the surface of SARS-CoV-2 virion. RBD of S can be in “down” and “up” conformation. Only the “up” conformation allows RBD to bind ACE2. Full-length ACE2 on cell membrane is cleaved by ADAM17 or TMPRSS2. After being shed by ADAM17, soluble ACE2 (sACE2) is released into extracellular side, which remains the enzymatic activity and the ability to bind SARS-CoV-2, can prevent the virus from invading host cells and spreading. The trimeric S is cleaved by furin or furin-like proprotein convertase into the S1 and S2 subunits during viral assembly in the infected cells. Binding to ACE2 on cell membrane induces the “up” conformation of RBD, which facilitates the shedding of the S1-ACE2 complex. S2 undergoes conformational changes to trigger the membrane fusion. S means spike protein; Ang means angiotensin. ACE2 dimer: red and wheat; B^oAT1 dimer: deep blue and violet; S trimer: cyan, gray and orange; ADAM17: blue; TMPRSS2: red-orange; furin: blue.

mechanism of SARS-CoV-2 infection and its symptom determinants to develop effective therapeutic and prophylactic strategies.

ACE2 and renin–angiotensin system

ACE2, encoded by a gene located on the X chromosome, was discovered in 2000 as a protein homolog of ACE [13,18]. Multiple studies have shown that ACE2 is highly expressed in many tissues, including the small intestine, thyroid, kidney, heart, testis and adipose tissue, and expressed at low levels in the blood, spleen, muscle, brain, and bone marrow [19–21]. ACE2 is a type I transmembrane (TM) glycoprotein with a full length of 805 amino acid residues that can be divided into two parts: the N-terminal catalytic domain (also known as the peptidase domain, PD) and the C-terminal collectrin-like domain (CLD) [13,22]. PD of ACE2, which shares 42% sequence homology with the N-terminal domain of ACE, contains a zinc ion in its active site. CLD of ACE2 contains a single TM helix and has approximately 48% sequence homology with collectrin, which does not contain a PD [22].

ACE2 plays an important role in the renin–angiotensin system [23,24] (Figure 1). Both ACE2 and ACE have peptidase activity, but their substrates and cleavage mechanisms are different [25]. ACE is a dipeptidyl peptidase that releases a dipeptide from the C-terminal of its substrate per digestion reaction, while ACE2 cleaves one amino acid. The crystal structure of ACE2 [25] shows that Arg273 forms a salt bridge with the C-terminal of the substrate. But in ACE, it is replaced with the smaller amino acid Glu, which explains the difference in substrate specificity between ACE and ACE2.

To be exact, ACE converts Ang I to Ang II, which function is to constrict blood vessels and raise blood pressure. ACE2 cleaves Ang II to Ang-(1–7), the role of which is to relax blood vessels and lower blood pressure. ACE2 can also convert Ang I into Ang-(1–9), and ACE or other peptidases will then convert Ang-(1–9) into Ang-(1–7) (Figure 1). ACE2 much more efficiently cleaves Ang II into Ang-(1–7) than Ang I into Ang-(1–9) [26]. Therefore, ACE2 plays a role in lowering blood pressure in the renin–angiotensin system. In

addition, Ang-(1–7) mediates various effects, including vasodilation, anti-inflammatory, anti-oxidation and so on [27], by binding the G protein-coupled receptor Mas [28], making Ang-(1–7) a promising therapeutic target for cardiovascular disease [29]. In summary, ACE2 negatively regulates the level of Ang II and maintains the balance with ACE to control local homeostasis to protect the lung, kidney, and cardiovascular system [14].

ACE2 and B⁰AT1 complex

ACE2 is reported to be a molecular chaperone of the neutral amino acid transporter B⁰AT1 in small intestine, which is also known as SLC6A19 that belongs to the neurotransmitter and amino acid co-transporter SLC6 family [16,30]. The *slc6a19* gene is located on the chromosome 5 and was cloned in 2004 because its mutation causes Hartnup disorder, an autosomal recessive condition that leads to aminoaciduria and eventually to symptoms like photosensitive rash, cerebellar ataxia and emotional instability [31–33]. It is also called B⁰AT1 due to the properties of system B⁰ which mediates the Na⁺-dependent neutral amino acid transporter [32]. B⁰AT1 has a total length of 634 amino acid residues, including its N-terminal and C-terminal on the intracellular side, as well as 12 TM helices arranged as a LeuT-fold [17]. The plasma membrane location of B⁰AT1 requires the chaperone of some proteins, represented by ACE2 in the small intestine or collectrin in the kidney [16,30].

It was reported that ACE2-B⁰AT1 complex was involved in immunoregulation by controlling amino acid homeostasis, antimicrobial peptide expression and ecological regulation of intestinal microbes [34–37]. Further research showed that this complex affects the composition of the gut microbiota through its role in amino acid transport, which may explain why amino acid malnutrition in Hartnup disease can lead to diarrhea and intestinal inflammation [35,36]. The *ace2* gene knockout mice showed a high susceptibility to intestinal inflammation and diarrhea, which could be reversed by dietary tryptophan or its metabolite nicotinamide, which are necessary for the biosynthesis of nicotinamide adenine dinucleotide (phosphate) via the kynurenine pathway [37]. The Hartnup diseases patients show symptoms of the skin and psychiatric disorders that are also ameliorated by nicotinamide supplementation [36]. The similarity between these studies is related to the weakened function of B⁰AT1 that transports neutral amino acids, suggesting that ACE2 is essential for the expression and stability of B⁰AT1 in the small intestine [16,30,37].

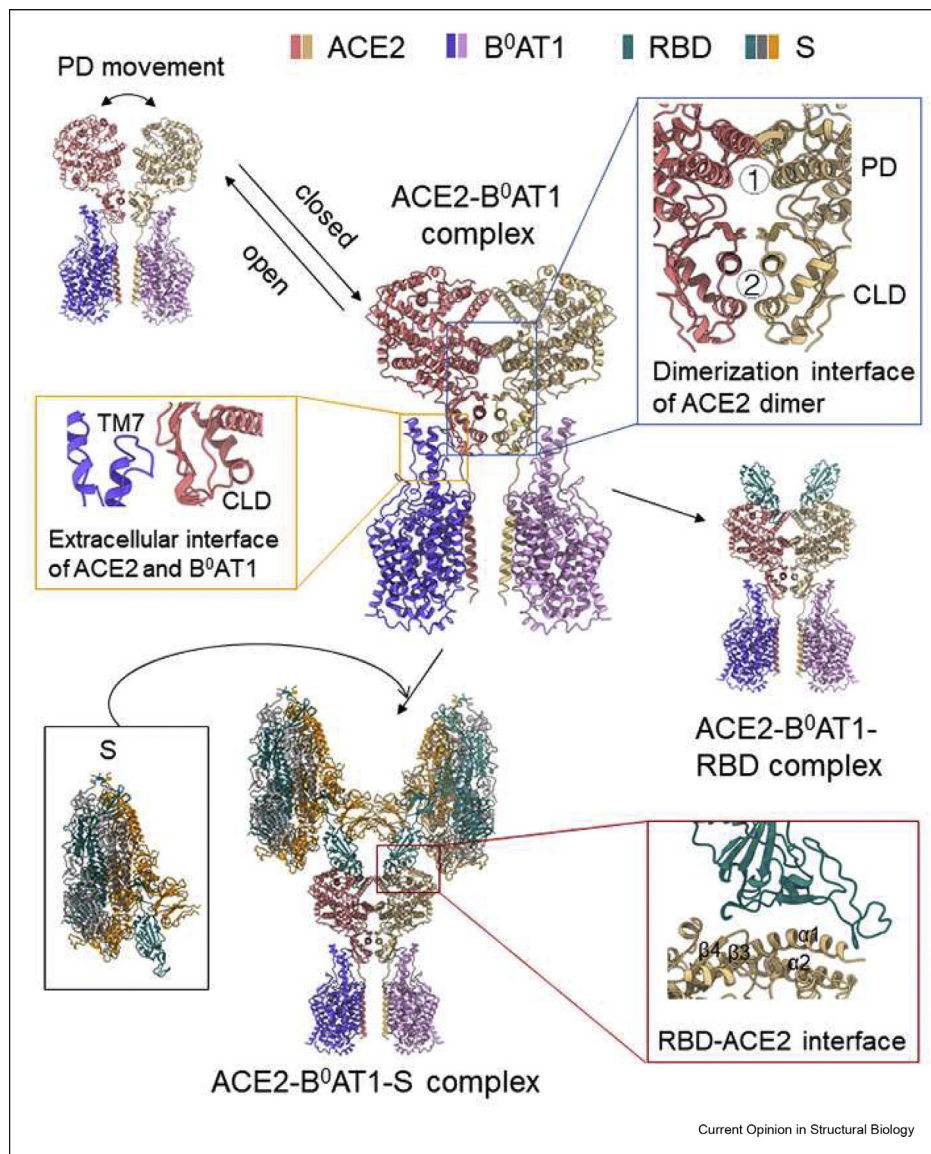
The high-resolution cryo-EM structure of the ACE2-B⁰AT1 complex revealed that ACE2 and B⁰AT1 form a heterodimer, and this heterodimer further forms a dimer through the ACE2-mediated dimerization interfaces

(Figure 2) [17]. ACE2 has two dimerization interfaces, one of which is mediated by weaker interactions in PD and can be disrupted, inducing a conformational change of ACE2 from a closed conformation to an open conformation [17]. Another interface is mediated by CLD with extensive polar interactions. The properties of the ACE2 dimeric interfaces suggest that ACE2 can form a dimer independently in the absence of B⁰AT1. Besides, B⁰AT1 adopts a typical LeuT-fold, whose TM7 helix region extends to the extracellular and binds CLD of ACE2 (Figure 2), suggesting that ACE2 can regulate the transport activity of B⁰AT1 through this interface.

ACE2 as SARS-CoV-2 receptor

ACE2 is the entry receptor for SARS-CoV-2 [5,7], as well as other coronaviruses such as SARS-CoV-1 [38] and NL63 [39]. The virion particles of SARS-CoV-2 are irregularly spherical with dozens of S randomly arranged on the surface, which can bind to ACE2 [17,40–42] and then bring viral genetic material into cells through membrane fusion at cell surface or later at endosome after endocytosis [6–9]. RBD of S has two conformations, “up” and “down” [1,2], of which only the “up” conformation can bind the receptor (Figure 1). A binding assay showed that SARS-CoV-2 binds ACE2 more strongly than SARS-CoV-1 does [1]. The interface between ACE2 and RBD of SARS-CoV-2 is similar to that of SARS-CoV-1, which is mainly involved in polar interactions. The extended loop region of RBD spans the α 1 helix of ACE2 like an arch bridge (Figure 2) [17]. Besides, the functional study and cryo-EM structure of the extracellular domain of S (S-ECD) and the ACE2-B⁰AT1 complex showed that each ACE2 monomer in the ACE2 dimer can bind an S (Figure 2) [17,43,44]. Structures of S of SARS-CoV-2 in different states in complex with ACE2 showed that PD of ACE2 binds S with a consistent interface and triggers the conformational change of S1 region to activate RBD. The uncleaved and trypsin-digested S-ECD alone exhibits an almost identical conformation, but the trypsin-digested S-ECD can be bound by more molecules of PD of ACE2 [44]. To be noticed, S has great structural flexibility and can form complex with ACE2 in various conformations [45,46]. RBD of SARS-CoV-2 variants tends to be in “up” state, so it more easily binds ACE2, which exhibits stronger infectivity. Interestingly, previous studies showed that the cleavage of the C-terminal segment of ACE2, especially residues 697 to 716, by proteases such as TMPRSS2 can enhance the S-driven viral entry [47,48]. In the ACE2-B⁰AT1 complex structure, the residues 697–716 of ACE2 form helices in CLD and map to the dimeric interface. The presence of B⁰AT1 might block the access of TMPRSS2 to the cutting site on ACE2 (Figure 1). These findings revealed the structural basis for the activation of S during infection

Figure 2



Cryo-EM structures of the ACE2-B⁰AT1 complex and their complex with RBD or S of SARS-CoV-2. The ACE2-B⁰AT1 complex exists as a dimer of heterodimer, with two dimerization interfaces mediated by PD (①) or CLD (②) of ACE2, respectively. The disruption of PD dimerization interface induces ACE2 changing from a closed state to an open state. CLD of ACE2 binds to TM7 of B⁰AT1 that extends to the extracellular. Each monomer in the dimeric ACE2 molecule can be bound by a RBD or an S of SARS-CoV-2. The binding interface between RBD of SARS-CoV-2 and ACE2 is mainly mediated by polar interactions, with the extended loop region of RBD spanning the α 1 helix of PD of ACE2 like a bridge. S means spike protein, RBD means receptor binding domain, TM means transmembrane, PD means peptidase domain and CLD means collectrin-like domain. ACE2 dimer is colored as red and wheat; B⁰AT1 dimer is colored as deep blue and violet; RBD is colored as cyan; S trimer is colored as cyan, gray, and orange.

and led to the development of specific peptide drugs [49] against SARS-CoV-2.

In addition, SARS-CoV-2 is a zoonotic pathogen, which can infect a variety of animals [50]. The cryo-EM structure of the complex of cat ACE2 and RBD of SARS-CoV-2 shows that cat ACE2 and human ACE2 bind the virus in a similar manner [50].

The different forms of ACE2

There are two forms of ACE2: the full-length form, which exists on the cell membrane, and the soluble form (sACE2), which is generated by enzymes cleavage [51]. ACE2 is cleaved by the metalloprotease ADAM17 [52] or the serine protease TMPRSS2, and then released into the blood. The cleavage patterns and functions of the two enzymes are different: only ACE2 cut by TMPRSS2

enhances SARS-CoV-1 infection [48]. Expression of TMPRSS2 inhibits the shedding of ACE2 by ADAM17. In many lung diseases, treatment with recombinant ACE2 can prevent blood vessel and lung damage. sACE2 has enzymatic activity and the ability to bind SARS-CoV-2, which can prevent the virus from invading host cells and spreading [53]. Recombinant sACE2 molecules can reduce viral load and prevent SARS-CoV-2 infection in blood vessels and kidney organoids [54]. An artificially designed trimeric ACE2 molecule eliminated the symmetry mismatch with trimeric S, and greatly enhanced the affinity between ACE2 and S [55]. The trimeric ACE2 molecule induces three RBDs of S to open state, which has an excellent ability to neutralize viruses [55]. Antibodies targeting to ACE2 can compete with viruses to bind ACE2, so they can be used for antiviral prevention and treatment [56]. However, a recent study found the secretory form of ACE2 can mediate the endocytosis of SARS-CoV-2 by the interaction between S and sACE2 or sACE2-vasopressin through AT1 or AVPR1B, respectively [57]. The concrete mechanism of soluble form of ACE2 requires further investigation.

Relationship with intestinal diseases

Gastrointestinal symptoms, including nausea, vomiting, anorexia, abdominal discomfort, diarrhea, are one class of the symptoms of COVID-19 infection [58,59], SARS-CoV-2 can be detected in the stool samples and rectal swabs of COVID-19 patients, suggesting the invasion of digestive tract by this virus [60,61]. These findings are supported by an assay for SARS-CoV-2 infection with human small intestinal organoids [62]. In addition, previous studies have shown that intestinal inflammation and diarrhea occur in *ace2* gene knockout mice and Hartnup disease patients caused by B⁰AT1 mutation [35–37]. These results collectively support the hypothesis that intestinal ACE2 engagement by S of SARS-CoV-2 might negatively regulate the absorption of neutral amino acids in the small intestine of COVID-19 patients, leading to diarrhea and intestinal inflammation. Other studies have shown that SARS-CoV-2 was detected in the small intestine, but small intestine infection appeared to have an attenuating effect on SARS-CoV-2-associated inflammation and a reduction in mortality in COVID-19 patients [59,63,64]. Further studies are required due to individual differences and limited case numbers.

Conclusion

ACE2 plays a major role in the renin–angiotensin system as a peptidase, and participates in the absorption and metabolism of amino acids as a molecular chaperone of B⁰AT1, thus related to the intestinal inflammation. Over the past two years, ACE2 has attracted much attention as the entry receptor of SARS-CoV-2. The physiological functions and the tissue expression and distribution of ACE2 are one of keys to understanding

the symptoms of COVID-19. The interaction interface between receptor and virus is an important target for developing drugs to inhibit viral invasion and alleviate infection symptoms. A variety of potential drugs to block virus binding receptors are being developed, including small molecules, peptides, and a variety of potent neutralizing antibodies. In addition, therapeutic strategies such as supplementation with essential amino acids, soluble ACE2 [53,54] or Ang-(1–7) [27–29] have been proposed and should be considered.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was funded by the National Natural Science Foundation of China (projects 32022037, 31971123), the Leading Innovative and Entrepreneur Team Introduction Program of Hangzhou, and the Special Research Program of Novel Coronavirus Pneumonia of Westlake University and Tencent Foundation. We would like to express our sincere gratitude towards the generous supports from Tencent Foundation and Westlake Education Foundation.

References

Papers of particular interest, published within the period of review, have been highlighted as:

- * of special interest
- ** of outstanding interest

1. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, Graham BS, McLellan JS: **Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.** *Science* 2020.
2. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D: **Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein.** *Cell* 2020, **181**:281–292. e286.
3. Weinstein RA: **Planning for epidemics—the lessons of SARS.** *N Engl J Med* 2004, **350**:2332–2334.
4. Hoffmann M, Kleine-Weber H, Pohlmann S: **A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells.** *Mol Cell* 2020, **78**:779–784 e775.
5. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, *et al.*: **A pneumonia outbreak associated with a new coronavirus of probable bat origin.** *Nature* 2020, **579**:270–273.
- This work reports the identification and characterization of SARS-CoV-2 and confirmed that SARS-CoV-2 uses the same cell entry receptor ACE2 as SARS-CoV.
6. Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F: **Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2.** *J Virol* 2010, **84**:12658–12664.
7. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, *et al.*: **SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor.** *Cell* 2020, **181**:271–280. e278.
- This work shows that SARS-CoV-2 uses ACE2 for cell entry and the serine protease TMPRSS2 for priming of the S protein. This work also shows a TMPRSS2 inhibitor approved for clinical use by blocking viral entry.
8. Simmons G, Gosalia DN, Rennekamp AJ, Reeves JD, Diamond SL, Bates P: **Inhibitors of cathepsin L prevent severe acute respiratory syndrome coronavirus entry.** *Proc Natl Acad Sci U S A* 2005, **102**:11876–11881.
9. Jackson CB, Farzan M, Chen B, Choe H: **Mechanisms of SARS-CoV-2 entry into cells.** *Nat Rev Mol Cell Biol* 2022, **23**:3–20.

10. Chi X, Yan R, Zhang J, Zhang G, Zhang Y, Hao M, Zhang Z, Fan P, Dong Y, Yang Y, *et al.*: **A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2.** *Science* 2020, **369**:650–655.
11. Yan R, Wang R, Ju B, Yu J, Zhang Y, Liu N, Wang J, Zhang Q, Chen P, Zhou B, *et al.*: **Structural basis for bivalent binding and inhibition of SARS-CoV-2 infection by human potent neutralizing antibodies.** *Cell Res* 2021, <https://doi.org/10.1038/s41422-021-00487-9>.
12. Wang F, Kream RM, Stefano GB: **Long-term respiratory and neurological sequelae of COVID-19.** *Med Sci Mon Int Med J Exp Clin Res* 2020, **26**, e928996.
13. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, *et al.*: **A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9.** *Circ Res* 2000, **87**:E1–E9.
14. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, *et al.*: **Angiotensin-converting enzyme 2 protects from severe acute lung failure.** *Nature* 2005, **436**: 112–116.
15. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, *et al.*: **A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury.** *Nat Med* 2005, **11**:875–879.
16. Kowalczyk S, Broer A, Tietze N, Vanslambrouck JM, Rasko JE, Broer S: **A protein complex in the brush-border membrane explains a Hartnup disorder allele.** *Faseb J* 2008, **22**:2880–2887.
17. Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q: **Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2.** *Science* 2020, **367**:1444–1448.
- This work reported the cryo-EM structures of full-length human ACE2 in complex with the amino acid transporter B⁰AT1, with or without the RBD domain of the spike protein of SARS-CoV-2. The collectrin-like domain of ACE2 mediates homo-dimerization of the ACE2-B⁰AT1 heterodimer. The extracellular peptidase domain of ACE2 is recognized by SARS-CoV-2 RBD mainly through polar residues. This work provides important insights to the structural basis for receptor recognition and infection of SARS-CoV-2.
18. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ: **A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase.** *J Biol Chem* 2000, **275**:33238–33243.
19. Hamming I, Timens W, Bultuis ML, Lely AT, Navis G, van Goor H: **Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis.** *J Pathol* 2004, **203**:631–637.
20. Hikmet F, Méar L, Å Edvinsson, Micke P, Uhlén M, Lindskog C: **The protein expression profile of ACE2 in human tissues.** *Mol Syst Biol* 2020, **16**, e9610.
21. Wang Y, Wang Y, Luo W, Huang L, Xiao J, Li F, Qin S, Song X, Wu Y, Zeng Q, *et al.*: **A comprehensive investigation of the mRNA and protein level of ACE2, the putative receptor of SARS-CoV-2, in human tissues and blood cells.** *Int J Med Sci* 2020, **17**:1522–1531.
22. Zhang H, Wada J, Hida K, Tsuchiyama Y, Hiragushi K, Shikata K, Wang H, Lin S, Kanwar YS, Makino H, Collectrin: **A collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys.** *J Biol Chem* 2001, **276**:17132–17139.
23. Xiao L, Sakagami H, Miwa N: **ACE2: the key molecule for understanding the pathophysiology of severe and critical conditions of COVID-19: demon or angel?** *Viruses* 2020:12.
24. Guney C, Akar F: **Epithelial and endothelial expressions of ACE2: SARS-CoV-2 entry routes.** *J Pharm Pharmacol Sci* 2021, **24**:84–93.
25. Towler P, Staker B, Prasad SG, Menon S, Tang J, Parsons T, Ryan D, Fisher M, Williams D, Dales NA, *et al.*: **ACE2 X-ray structures reveal a large hinge-bending motion important for inhibitor binding and catalysis.** *J Biol Chem* 2004, **279**: 17996–18007.
26. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, *et al.*: **Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase.** *J Biol Chem* 2002, **277**:14838–14843.
27. Rodrigues Prestes TR, Rocha NP, Miranda AS, Teixeira AL, Simoes ESAC: **The anti-inflammatory potential of ACE2/angiotensin-(1-7)/mas receptor Axis: evidence from basic and clinical research.** *Curr Drug Targets* 2017, **18**:1301–1313.
28. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M, *et al.*: **Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas.** *Proc Natl Acad Sci U S A* 2003, **100**:8258–8263.
29. Chamsi-Pasha MA, Shao Z, Tang WH: **Angiotensin-converting enzyme 2 as a therapeutic target for heart failure.** *Curr Heart Fail Rep* 2014, **11**:58–63.
30. Camargo SM, Singer D, Makrides V, Huggel K, Pos KM, Wagner CA, Kuba K, Danilczyk U, Skovby F, Kleta R, *et al.*: **Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with hartnup mutations.** *Gastroenterology* 2009, **136**:872–882.
31. Seow HF, Broer S, Broer A, Bailey CG, Potter SJ, Cavanaugh JA, Rasko JE: **Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter SLC6A19.** *Nat Genet* 2004, **36**:1003–1007.
32. Bröer A, Klingel K, Kowalczyk S, Rasko JE, Cavanaugh J, Bröer S: **Molecular cloning of mouse amino acid transport system B0, a neutral amino acid transporter related to Hartnup disorder.** *J Biol Chem* 2004, **279**:24467–24476.
33. Kleta R, Romeo E, Ristic Z, Ohura T, Stuart C, Arcos-Burgos M, Dave MH, Wagner CA, Camargo SR, Inoue S, *et al.*: **Mutations in SLC6A19, encoding B0AT1, cause Hartnup disorder.** *Nat Genet* 2004, **36**:999–1002.
34. Benigni A, Cassis P, Remuzzi G: **Angiotensin II revisited: new roles in inflammation, immunology and aging.** *EMBO Mol Med* 2010, **2**:247–257.
35. Camargo SMR, Vuille-Dit-Bille RN, Meier CF, Verrey F: **ACE2 and gut amino acid transport.** *Clin Sci (Lond)* 2020, **134**:2823–2833.
36. Nisoli E, Cinti S, Valerio A: **COVID-19 and Hartnup disease: an affair of intestinal amino acid malabsorption.** *Eat Weight Disord* 2021, **26**:1647–1651.
37. Hashimoto T, Perlot T, Rehman A, Trichereau J, Ishiguro H, Paolino M, Sigl V, Hanada T, Hanada R, Lipinski S, *et al.*: **ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation.** *Nature* 2012, **487**:477–481.
38. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, *et al.*: **Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus.** *Nature* 2003, **426**:450–454.
39. Hofmann H, Pyrc K, van der Hoek L, Geier M, Berkhout B, Pohlmann S: **Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry.** *Proc Natl Acad Sci U S A* 2005, **102**:7988–7993.
40. Wang Q, Zhang Y, Wu L, Niu S, Song C, Zhang Z, Lu G, Qiao C, Hu Y, Yuen KY, *et al.*: **Structural and functional basis of SARS-CoV-2 entry by using human ACE2.** *Cell* 2020, **181**:894–904. e899.
- This work reported the crystal structure of the RBD of SARS-CoV-2 S in complex with the peptidase domain of ACE2 and provided important clues for initial infection.
41. Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L, *et al.*: **Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor.** *Nature* 2020, **581**:215–220.
- This work reported the crystal structure of the RBD of SARS-CoV-2 S in complex with the peptidase domain of ACE2 at a high resolution and provided important clues for initial infection.
42. Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F: **Structural basis of receptor recognition by SARS-CoV-2.** *Nature* 2020, **581**:221–224.

This work reported the crystal structure of the RBD of SARS-CoV-2 S in complex with the peptidase domain of ACE2 and provided important clues for initial infection.

43. Stevens BR, Ellory JC, Preston RL: **B0AT1 amino acid transporter complexed with SARS-CoV-2 receptor ACE2 forms a heterodimer functional unit: in situ conformation using radiation inactivation analysis.** *Function* 2021, **2**.
44. Yan R, Zhang Y, Li Y, Ye F, Guo Y, Xia L, Zhong X, Chi X, Zhou Q: **Structural basis for the different states of the spike protein of SARS-CoV-2 in complex with ACE2.** *Cell Res* 2021, <https://doi.org/10.1038/s41422-021-00490-0>.
This work reported cryo-EM structures of the S protein of SARS-CoV-2 at multiple conformations or states, including locked, activated, S1/S2 trypsin-cleaved, peptidase domain (PD) of ACE2-bound, and the full-length ACE2-bound states. The protease-processed S protein tends to accommodate more PD of ACE2. The structure of the S/ACE2/B⁰AT1 ternary complex indicates that one ACE2 dimer can be bound by two trimeric S proteins simultaneously. This work provides clues for the activation mechanism of the S protein of SARS-CoV-2.
45. Benton DJ, Wrobel AG, Xu P, Roustan C, Martin SR, Rosenthal PB, Skehel JJ, Gamblin SJ: **Receptor binding and priming of the spike protein of SARS-CoV-2 for membrane fusion.** *Nature* 2020, <https://doi.org/10.1038/s41586-020-2772-0>.
This work analyzed the binding of ACE2 to the furin-cleaved spike protein of SARS-CoV-2 using cryo-EM and classified multiple molecular species, describing ACE2-binding events during the priming and the activation of the S protein.
46. Xu C, Wang Y, Liu C, Zhang C, Han W, Hong X, Wang Y, Hong Q, Wang S, Zhao Q, *et al.*: **Conformational dynamics of SARS-CoV-2 trimeric spike glycoprotein in complex with receptor ACE2 revealed by cryo-EM.** *Sci Adv* 2021, **7**.
47. Shulla A, Heald-Sargent T, Subramanya G, Zhao J, Perlman S, Gallagher T: **A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry.** *J Virol* 2011, **85**:873–882.
48. Heurich A, Hofmann-Winkler H, Gierer S, Liepold T, Jahn O, Pohlmann S: **TMPRSS2 and ADAM17 cleave ACE2 differentially and only proteolysis by TMPRSS2 augments entry driven by the severe acute respiratory syndrome coronavirus spike protein.** *J Virol* 2014, **88**:1293–1307.
49. Wang G, Yang ML, Duan ZL, Liu FL, Jin L, Long CB, Zhang M, Tang XP, Xu L, Li YC, *et al.*: **Dalbavancin binds ACE2 to block its interaction with SARS-CoV-2 spike protein and is effective in inhibiting SARS-CoV-2 infection in animal models.** *Cell Res* 2021, **31**:17–24.
This work discovered dalbavancin, a lipoglycopeptide antibiotic, as a promising anti-COVID-19 drug candidate based on virtual screening. Dalbavancin directly binds to ACE2 with high affinity, blocking SARS-CoV-2 replication with an EC₅₀ in nM range.
50. Wu L, Chen Q, Liu K, Wang J, Han P, Zhang Y, Hu Y, Meng Y, Pan X, Qiao C, *et al.*: **Broad host range of SARS-CoV-2 and the molecular basis for SARS-CoV-2 binding to cat ACE2.** *Cell Discov* 2020, **6**:68.
51. Battle D, Wysocki J, Satchell K: **Soluble angiotensin-converting enzyme 2: a potential approach for coronavirus infection therapy?** *Clin Sci (Lond)* 2020, **134**:543–545.
52. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, Hooper NM, Turner AJ: **Tumor necrosis factor- α convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2).** *J Biol Chem* 2005, **280**:30113–30119.
53. Chan KK, Tan TJC, Narayanan KK, Procko E: **An engineered decoy receptor for SARS-CoV-2 broadly binds protein S sequence variants.** *Sci Adv* 2021, **7**.
54. Monteil V, Kwon H, Prado P, Hagelkruys A, Wimmer RA, Stahl M, Leopoldi A, Garreta E, Hurtado Del Pozo C, Prosper F, *et al.*: **Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2.** *Cell* 2020, **181**:905–913. e907.
55. Guo L, Bi W, Wang X, Xu W, Yan R, Zhang Y, Zhao K, Li Y, Zhang M, Cai X, *et al.*: **Engineered trimeric ACE2 binds viral spike protein and locks it in "Three-up" conformation to potentially inhibit SARS-CoV-2 infection.** *Cell Res* 2020, <https://doi.org/10.1038/s41422-020-00438-w>.
This work engineered a trimeric ACE2 protein (T-ACE2) that could bind spike protein with extremely high affinity to potentially inhibit infection of coronaviruses that utilize ACE2 as receptor, including SARS-CoV-2, SARS-CoV-2 mutants, and SARS-CoV. T-ACE2 can avoid the mutational escape and could also be used to treat the virus-infected patients or detect all SARS-CoVs and related viruses.
56. Chen Y, Zhang YN, Yan R, Wang G, Zhang Y, Zhang ZR, Li Y, Ou J, Chu W, Liang Z, *et al.*: **ACE2-targeting monoclonal antibody as potent and broad-spectrum coronavirus blocker.** *Signal Transduct Targeted Ther* 2021, **6**:315.
This work reported a human ACE2-targeting monoclonal antibody, 3E8, which blocked infection of multiple coronaviruses including wild type and mutant SARS-CoV-2, SARS-CoV and HCoV-NL63. Cryo-EM structure of ACE2 in complex with 3E8 revealed an anti-coronavirus epitope on human ACE2. This work provided a potent and broad-spectrum strategy against all coronaviruses that target ACE2 as receptor.
57. Yeung ML, Teng JLL, Jia LL, Zhang CY, Huang CX, Cai JP, Zhou RH, Chan KH, Zhao HJ, Zhu L, *et al.*: **Soluble ACE2-mediated cell entry of SARS-CoV-2 via interaction with proteins related to the renin-angiotensin system.** *Cell* 2021, **184**:2212 [–+].
58. Song Y, Liu P, Shi XL, Chu YL, Zhang J, Xia J, Gao XZ, Qu T, Wang MY: **SARS-CoV-2 induced diarrhoea as onset symptom in patient with COVID-19.** *Gut* 2020, **69**:1143–1144.
59. Guo M, Tao W, Flavell RA, Zhu S: **Potential intestinal infection and faecal-oral transmission of SARS-CoV-2.** *Nat Rev Gastroenterol Hepatol* 2021, **18**:269–283.
60. Xu Y, Li X, Zhu B, Liang H, Fang C, Gong Y, Guo Q, Sun X, Zhao D, Shen J, *et al.*: **Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding.** *Nat Med* 2020, **26**:502–505.
61. Chen Y, Chen L, Deng Q, Zhang G, Wu K, Ni L, Yang Y, Liu B, Wang W, Wei C, *et al.*: **The presence of SARS-CoV-2 RNA in the feces of COVID-19 patients.** *J Med Virol* 2020, **92**:833–840.
62. Lamers MM, Beumer J, van der Vaart J, Knoops K, Puschhof J, Breugem TI, Ravelli RBG, van Schayck JP, Mykytyn AZ, Duimel HQ, *et al.*: **SARS-CoV-2 productively infects human gut enterocytes.** *Science* 2020, **369**:50 [–+].
This work demonstrated enterocytes were infected by SARS-CoV and SARS-CoV-2 in human small intestinal organoids.
63. Lehmann M, Allers K, Heldt C, Meinhardt J, Schmidt F, Rodriguez-Sillke Y, Kunkel D, Schumann M, Bottcher C, Stahl-Hennig C, *et al.*: **Human small intestinal infection by SARS-CoV-2 is characterized by a mucosal infiltration with activated CD8(+) T cells.** *Mucosal Immunol* 2021, **14**:1381–1392.
64. Livanos AE, Jha D, Cossarini F, Gonzalez-Reiche AS, Tokuyama M, Aydlilo T, Parigi TL, Ladinsky MS, Ramos I, Dunleavy K, *et al.*: **Intestinal host response to SARS-CoV-2 infection and COVID-19 outcomes in patients with gastrointestinal symptoms.** *Gastroenterology* 2021, **160**:2435–2450. e2434.